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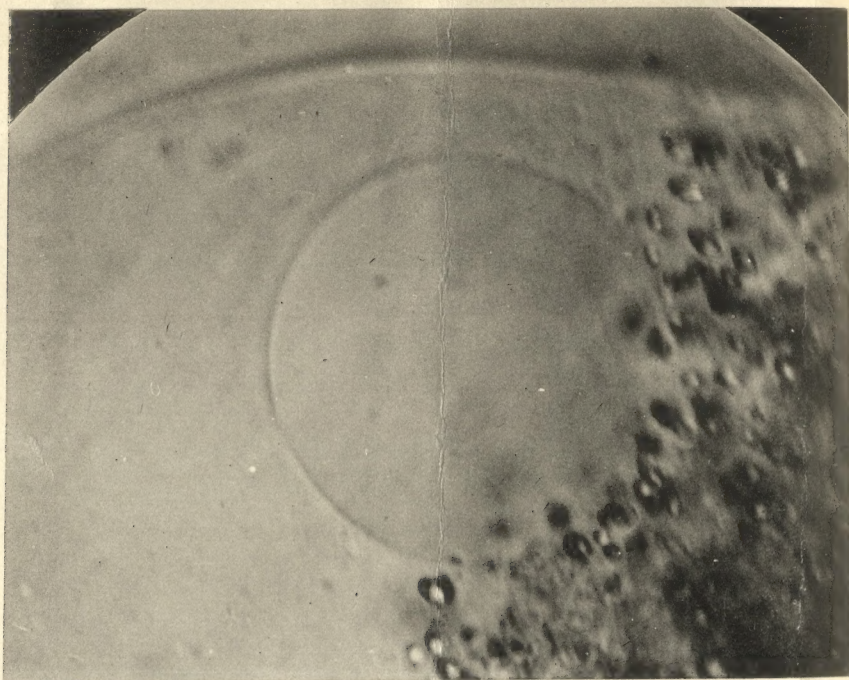


Fig. 1. Contractile Vacuole of Amoeba.

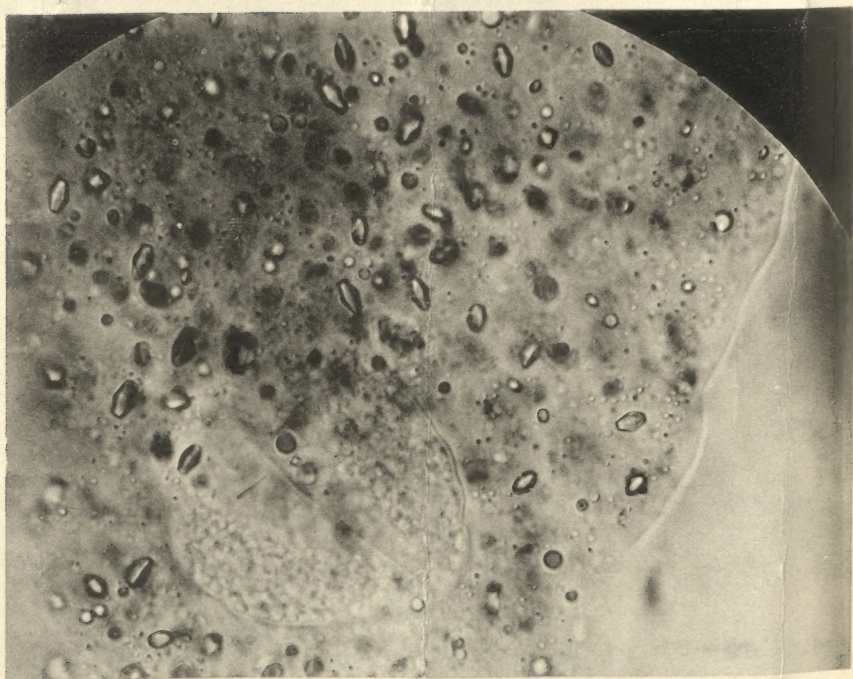


Fig. 2. Crystalline Bodies, Edge of Ectosarc, and Nucleus.

PHOTOMICROGRAPHS OF AMOEBA PROTEUS; OBLIQUE LIGHT; X 1000 DIAMETER.



## THE REACTION OF AMŒBA TO LIGHTS OF DIFFERENT COLORS.

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A NUMBER of the lower plants are able to distinguish lights of different intensities. Others, such as the purple bacteria and swarm-spores, have been shown to respond in different ways to differently colored lights of presumably the same degree of intensity. The purpose of this paper is to show that the common *Amœba proteus* possesses both these kinds of sensitiveness to light; its protoplasm may be set in motion or brought to rest by varying the light to which it is exposed. Further it will be shown that the response is quicker to some light rays than to others. Finally, certain observations on the structure and the contents of the cytoplasm and on the nature of streaming will be discussed.

Without claiming new methods of work, it may be pointed out that the use of the apparatus hereafter described and the employment of colored screens to promote streaming, enables us to approach from a favorable standpoint the difficult problem of the nature and meaning of protoplasmic flow. Light is easily controlled, and its action can be observed throughout the whole or in any part of the transparent organism.

**The effect on streaming of lights of different colors. Method of work.**—The amœbæ,<sup>1</sup> which for a research of this kind must be abundant and large, were studied from the image projected on the

<sup>1</sup> The amœbæ were procured for us from some decaying *Nitella* by the late Professor J. I. Peck, in Williamstown, Mass. While attempting to photograph one of the more active of these amœbæ, it was found that by throwing bright white light upon the field, the animal could be held in any phase of pseudopodial formation long enough for the securing of a photograph. The rigor thus produced was immediately relieved by red, green or violet light. This discovery led to the investigation here reported.

The photography, arrangement, and manipulation of apparatus were entirely the work of Edward Leaming, and were done in his laboratory in the Department of Pathology, Columbia University. The remainder of the work was done by N. R. Harrington.



ground glass back of a large Zeiss photomicrographic apparatus by means of an electric arc lamp. The change in the color of the light was effected almost instantaneously by the interposition of the light-filters of colored celloidin used by Bierstadt in photographing colors. Although these screens do not give purely monochromatic light, there is always one predominant color in the field, and there is no reason to suppose that the very striking results produced are complicated much by a slight mixture of rays different from that of the principal color, such as the slight red transmitted by the violet. To satisfy any doubts in this respect, the experiments have been repeated, using Hartnack's illuminating apparatus for monochromatic light, and it has been found that the same results are obtained with monochromatic and orthochromatic lights. It was, however, impossible to determine latent periods with the former light because of the difficulty in changing instantly from one part of the spectrum to another. It would have been desirable to work with sunlight and monochromatic screens, were an apparatus procurable which would give a colored light as brilliant as that obtained by an arc lamp and colored screens.<sup>1</sup>

A close comparison of the effect of pure and mixed colors shows that the slight impurities just discussed, for example, the faint red transmitted by the violet (to take the most striking case), are far less misleading than other factors which can never be entirely controlled. Such inaccessible factors are variability in moisture, oxygen, pressure, and capillary currents. It is necessary to take great care that temperature and intensity do not vary under the different color screens used.<sup>2</sup>

To show that variation in temperature was not a source of error, a delicate thermometer was suspended with the bulb in the position usually occupied by the amœba. After exposure for a considerable time to the hottest white light used by us, the mercury rose from 20.9°C., the temperature of the room, to 25.2°C. When celloidin films were interposed, the temperature settled to 24.8°C., and long exposures to red, green, and violet lights showed that no one of these films transmitted more heat than another. In order to make the temperature of the white uniform with that of the colored rays, a piece of mica was used with the former light.

<sup>1</sup> Since the above was written, the experiments described in the present article have been repeated with the spectrum, and results entirely confirmatory of those above described have been obtained.

<sup>2</sup> Jarring alone produced no perceptible constant effect.



It is very difficult to compare accurately the intensities of *transmitted* lights of different colors. The films might have been compared by Whitman's modification of the flicker method<sup>1</sup> had the process been known to the experimenters at the time. Screens were selected, however, that illuminated about equally an opaque screen and an observation was made which proved conclusively that the decisive factor is color, and not relative light or darkness. When an amæba is flowing rapidly, one violet screen tends to retard the flow. If now a second violet screen be inserted, still greater retardation is produced. The retardation cannot be due to the diminished intensity or brightness, for the normal effect of darkness after streaming is the resumption of streaming. The same can be proved in another way: — A yellow glass, which transmits rays of great intensity, will nevertheless allow the resumption of streaming after quiescence in violet. When the bright yellow is changed to a violet of much less brilliancy, streaming stops. Here is an instance of greater darkness and coincident retardation of flow. These facts show that in these cases color is a stronger factor than intensity.

Intensity, however, is a very important factor in phototaxis as is shown by the active streaming which often takes place during darkness, as well as by the retarding effect of very bright light. The fact that two green screens produce greater flow than one might be accounted for by the lessened intensity or the effect of the color.

The following typical experiment illustrates the method: —

An amæba of spherical form was brought into place on the camera. It remained in the dark field a considerable time without perceptible movement. From darkness a red light was suddenly thrown on. In ten seconds, a movement was apparent in the inner cytoplasm and one crystal after another began to change its position. A current of these particles and of the ground-substance in which they were suspended finally became established, and a pseudopod of the entire width of the body was formed, flowing rapidly across the field and followed by the remaining parts of the body. The organism was kept in red for two minutes and the rapid flow was maintained. When the red film was replaced by a violet one, the streaming slowed instantly, and in five seconds there was a complete stop. Screens of different colors were successively employed. Whenever green or red films were inserted the movement started. It was checked by violet or white light. In two or three instances after a change from red to violet there was not only cessation of flow, but a reversal of the current to an exactly opposite direction. It hap-

<sup>1</sup> WHITMAN: Physical review, 1896, iii, p. 241.



pened once or twice that after quite long exposure a current became established under violet light. This could be stopped only by more violet or white light. Occasionally a movement started under mild white light and this was instantly checked by changing the position of the condenser so as to increase the intensity. Under the bright white light produced in this way, the amœba usually assumed a tense rigor-like condition, but instead of a return to the spherical form, as might have been expected, the flowing was stopped instantly and that form of the body was retained which the amœba showed when the brightest light was first flashed upon it. Even pseudopodia which were just starting or those which had attained considerable impetus, were often checked instantly. This light-rigor is doubtless identical with the light-rigor demonstrated by Engelmann<sup>1</sup> in bacteria and by Pringsheim<sup>2</sup> in *Nitella*. On the other hand, it was observed that when the organism was left under white light for some time, a very manifest spasmodic attempt to form pseudopodia occurred. Ordinarily this effort was not accomplished, it being strongly suggested that restraining and impelling forces were acting at the same time.

**Experimental data.** — The observations which we have made will be presented here in a condensed form. A few preliminary remarks and examples will make it easy to follow them.

In white light the amœba usually appears tense, and as a rule no streaming of its protoplasm can be observed. When the light is changed to red, green, yellow, or even violet, streaming begins, after an interval or latent period. Sometimes the streaming is seen even in white light; it is then modified by the change to colored light. Similarly, the change from red to violet, or the reverse, increases or diminishes the protoplasmic flow. If the excitant be increased, by the use of a double color screen, the streaming may increase until the maximum is reached. The latent period differs with different colors. After white light, red will be followed by streaming sooner than will green. The time necessary for a given color to produce its characteristic effect is an index of the efficiency of the color in causing or retarding flow. It gives also the relative values of the colors with reference to their approach to the optimum color.

*Mild white following bright white* — streaming continues and is accelerated.

*Violet following white* : —

- (1) Flow started and continued for three minutes.
- (2) Pre-existing flow at first slowed, then started with a rush.

<sup>1</sup> ENGELMANN: Archiv f. d. ges. Physiol., 1879, xix, p. 1.

<sup>2</sup> PRINGSHEIM: Sitz.-Ber. der Akad. der Wissensch., Berlin, 1881, p. 504.



- (3) Flow started after fifteen seconds.
- (4) Pre-existing flow slightly lessened.
- (5) Flow started after one second.
- (6) Flow started after fifteen seconds.
- (7) Flow started and pseudopodia made in eight seconds.

*Green following white : —*

- (1) Flow started from pre-existing rest almost instantly.
- (2) Flow started from pre-existing rest almost instantly.
- (3) Flow started from pre-existing rest almost instantly.
- (4) Flow started from pre-existing rest after ten seconds.
- (5) Pre-existing flow stopped.
- (6) Slow pre-existing flow increased in thirty seconds.

*Yellow following white : —*

- (1) Flow started from pre-existing quiescence ; (repeated many times).
- (2) Pre-existing flow in one direction changed to flow in opposite direction.

*Red following white : —*

- (1) Diffuse flow started after few seconds.
- (2) Flow started instantly after quiescence ; (repeated many times).

*White following violet : —*

- (1) Momentary stop of pre-existing flow.
- (2) Instantaneous stop of pre-existing flow.
- (3) Increase of pre-existing flow.
- (4) Pre-existing flow stopped in thirty seconds.
- (5) Distinct slowing of pre-existing flow after ten seconds.
- (6) Increase of pre-existing flow after thirty seconds.

*White following green : —*

- (1) Pre-existing flow stopped ; (this was repeated many times and demonstrated to several observers).
- (2) Pre-existing flow in one direction stopped instantly and reversed.
- (3) After pre-existing quiescence flow started in two seconds ; (observed only once).

*White following yellow : —*

- (1) Pre-existing flow stopped almost instantly ; (observed several times).

*White following red : —*

- (1) Pre-existing flow stopped instantly ; (repeated many times).
- (2) Pre-existing flow stopped in thirty seconds.
- (3) Pre-existing flow stopped in three seconds.
- (4) Pre-existing flow stopped in five seconds.
- (5) Pre-existing flow stopped in three seconds.
- (6) Pre-existing flow stopped in six seconds.

} mild white.

} bright white.



*Violet following green : —*

- (1) Pre-existing flow stopped instantly ; (observed several times).
- (2) Pre-existing flow checked and reversed.
- (3) Pre-existing flow reversed in fifty-six seconds.
- (4) Pre-existing flow stopped in twenty seconds.
- (5) Pre-existing flow stopped in twenty-five seconds.

*Violet following yellow : —*

- (1) Pre-existing flow stopped in twenty-four seconds.

*Violet following red : —*

- (1) Pre-existing flow stopped and reversed.
- (2) Pre-existing flow checked.
- (3) Pre-existing flow stopped in forty seconds.
- (4) Pre-existing flow stopped in forty seconds.
- (5) Pre-existing flow stopped in five seconds.

*Deep blue following violet : —*

- (1) Pre-existing flow slowed instantly.
- (2) Pre-existing flow slowed after short time.
- (3) Pre-existing flow stopped.

*Green following violet : —*

- (1) Internal flow at once started.
- (2) Flow started from pre-existing quiescence in fifty-six seconds.
- (3) Flow started from pre-existing quiescence in sixteen seconds.
- (4) Flow started almost instantly ; (repeated many times).

*Yellow following violet : —*

- (1) No effect after pre-existing quiescence.
- (2) Flow started after ten seconds.
- (3) Instant increase of pre-existing flow.
- (4) Increase of pre-existing flow in one second.

*Red following violet : —*

- (1) Pre-existing flow in violet reversed in two seconds.
- (2) Flow started in three seconds.
- (3) Flow started in three seconds.
- (4) Flow started instantaneously.

*Purple (yellow and violet) following violet : —*

Pre-existing flow increased.

*Violet following purple (yellow and violet) : —*

Pre-existing flow stopped in three seconds.

Green, red, and yellow lights have so nearly similar effects on the organism that there is generally no perceptible change in streaming



when these three colors are interchanged. Nevertheless, that red is the most powerful excitant to flow is indicated by the shorter latent period after quiescence in white light.

Some of the recorded actions under two colors, both from the red end of the spectrum, are: —

*Green following yellow: —*

- (1) Pre-existing flow stopped after twenty-four seconds and started in opposite direction after twelve seconds more.
- (2) Pre-existing flow changed to diffuse flow in other directions.

*Yellow following green: —*

- (1) No perceptible effect.

*Green following red: —*

- (1) No perceptible change.
- (2) Pre-existing flow changed to another direction.
- (3) No perceptible change.
- (4) No perceptible change.
- (5) No perceptible change.

*Red following green: —*

- (1) Increase of pre-existing flow.
- (2) No perceptible change.
- (3) No perceptible change.

*Yellow following red: —*

- (1) No perceptible change.
- (2) No perceptible change.
- (3) Slight increase of pre-existing flow.

*Red following yellow: —*

- (1) No perceptible change.

These results can be conveniently summarized in the following table, which gives an average latent period for the effects of each individual color as an excitant and as a retarder.<sup>1</sup> The value of the average, it should be stated, is somewhat impaired by the difficulty of fixing the exact moment at which the effect begins to show itself or reaches its maximum. The numerals in the table indicate the interval in seconds between the application of the color and the production of its characteristic effect. The sign + indicates that the particular sequence of colors allows streaming to begin or, if already present, to increase; similarly, the sign — indicates that streaming is

<sup>1</sup> These terms are employed in this paper simply for the sake of brevity and convenience; the cause of protoplasmic flow is still an unsettled problem.



stopped or retarded in the given number of seconds. The asterisk shows that the change of color has no perceptible effect.

	White Preceding.	Violet Preceding.	Green Preceding.	Yellow Preceding.	Red Preceding.
White following . . *		-5 ?	-1	-1	-1
Violet following . . +5		*	-20	-24	-9
Green following . . +5		+12	*	? +12	*
Yellow following . . +1		+3	*	*	*
Red following . . . +1		+2	*	*	*

The table shows that the effectiveness of the following kinds of light as inhibitors of protoplasmic flow diminishes in the order named: white, violet, red. No experiments have been made in which the amœba has been long exposed to any one light for the purpose of ascertaining what particular wave-lengths are favorable to protoplasmic streaming. All the data collected above refer to the effects produced *by suddenly changing* five particular screens. In this connection, it should be stated that after a few minutes, streaming will commence under any light, if the amœba be a fairly active individual. An interesting problem lies open to the observer regarding the relative times which elapse before an amœba becomes attuned to lights of different colors.

The latent periods for two colors as sequent and precedent do not correspond. For instance, when yellow follows violet, streaming will start in three seconds; but when violet follows yellow, there is an interval of twenty-four seconds before any effect is observed. The effect, therefore, of yellow following violet is not exactly opposite to that of violet following yellow.

**Reaction of an enucleated fragment.** — If the water supply becomes partially exhausted, the amœba is apt to rupture and to pour out a large number of crystals with the surrounding ground-substance and oftentimes the nucleus. If water were added after the nucleus was extruded, it was found in one or two cases that the enucleated part seemed to heal rapidly and showed a faint streaming. Under red light the streaming was materially increased, but under white or violet suddenly checked. This reaction was obtained several times and the enucleated fragment survived three or four hours.

**Structure and contents of the cytoplasm.** — The granules in amœba, which have been described by Leidy and others as crystalline in structure, are constantly undergoing a vibration which suggests Brownian movement. This appearance is seen best under a very



high power. Under the high power it also may be observed that the ground-substance of amœba apparently contains a great number of small bodies which appear like active bacteria. As described by Leidy, the crystals are of several forms, the most prevalent being either barrel-shaped or spherical.

In the use of the apparatus above described, several opportunities were offered for observations on the structure of this protozoan. Some of these points are illustrated by the accompanying photographs of living amœba, which give interesting and vivid representations of protoplasm under a 1.5 mm. oil immersion objective. Fig. 1, Plate I, shows a contractile vacuole surrounded by homogeneous ground-substance. The homogeneous outer layer, generally called the ectosarc (ectoplasm), can be distinguished from the inner portions of the amœba only by the absence of granules. That this distinction is a purely arbitrary one is indicated by the specimens sometimes observed in which the granules are all aggregated at one end, leaving an entire end of the amœba composed of a translucent, homogeneous mass. The transparent half of one amœba of this kind was observed to contain seven large water (or contractile) vacuoles.

Since the granules may be carried by internal currents into all parts of the ground-substance, there is clearly no fundamental difference between the "clear peripheral substance, the ectoplasm, and a central substance, the entoplasm, filled with coarse granules."<sup>1</sup> If it is necessary to use the terms ectoplasm and entoplasm, the latter must be defined as that part of the general ground-substance in which crystals and granules are present, but it should be remembered that neither they nor the outer ground-substance are constant in position, for, as has been stated by Ryder and others, the inner and outer parts of amœba constantly interchange.

Figure 2, Plate I, shows the crystalline bodies, the edge of the ectosarc, and the nucleus.

**Nature of streaming.** — Streaming does not invariably seem to originate with or be controlled by that part of the protoplasm which is near the circumference. On the contrary, we have observed it to begin always near the centre of a mass of particles midway from either edge. This point may or may not coincide with the centre of the amœba itself. When an excitant light falls upon an amœba which is at rest, the first movement observed is often the shifting in

<sup>1</sup> SEDGWICK and WILSON: General biology.



position of one or a few granules. These are followed by others until a general current is established, the clear substance always preceding in the formation of the pseudopodia. When fully established, the streaming of the particles proceeds rapidly in the centre of the mass, but much less rapidly near the edges.

Although we have been able by means of the apparatus used in the prosecution of these experiments to study the formation of pseudopodia under the most favorable optical conditions possible, with an effective and easily controlled means of stimulation, we have not been able to contribute to the difficult question of the homology of protoplasmic streaming with muscle-contraction. No evidence has appeared which confirms Verworn's theory that the spherical form of *amœba* corresponds to the phase of full contraction in a muscle-fibre. The spherical form is especially characteristic of inert *amœbæ*, but colors which produce a cessation of streaming do not cause the assumption of the spherical form. It often occurs that in an environment which is not favorable to streaming (intense light or heat) the *amœba* assumes a spherical shape, but, if protoplasmic movement be checked by retardant light, the organism is most apt to stop in just that position in which it happens to be, and to remain so, indefinitely.

#### SUMMARY.

1. *Amœba* streams in the presence of red light.
2. Streaming is retarded, stopped, or reversed by rays from the violet end of the spectrum.
3. Further, the effectiveness of the following kinds of light as inhibitors of protoplasmic flow diminishes in the order named: white, violet, red.
4. Enucleated *amœbæ* stream in red light, and cease to stream in violet or white light.
5. No confirmation for the theory that the circular form of *Amœba* represents full contraction has been found. It still remains to be proved whether or not pseudopodia are produced or retracted by contractions — local or general — of the outer portion (ectoplasm).